



King's Research Portal

DOI:

[10.1016/j.jpsychires.2019.09.010](https://doi.org/10.1016/j.jpsychires.2019.09.010)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Schmidt, F. M., Koch, J., Nowak, C., Holdt, L. M., Teupser, D., Hegerl, U., & Himmerich, H. (2019). Ligands and receptors of the TNF superfamily are decreased in major depression and during early antidepressant therapy. *Journal of psychiatric research*, 119(0), 116-121. <https://doi.org/10.1016/j.jpsychires.2019.09.010>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Ligands and receptors of the TNF superfamily are decreased in major depression and during early antidepressant therapy

Authors: Frank M. Schmidt ^{1,#}, Jenny Koch¹, Claudia Nowak¹, Lesca M. Holdt², Daniel Teupser², Ulrich Hegerl¹, Hubertus Himmerich³

¹ Department of Psychiatry and Psychotherapy, University Hospital Leipzig, Semmelweisstr. 10, D-04103 Leipzig, Germany

² Institute of Laboratory Medicine, University Hospital Ludwig-Maximilian-University Munich, Munich, Germany

³ Department of Psychological Medicine, King's College London, London, UK

Corresponding author: Dr. med. Frank M. Schmidt
Department of Psychiatry and Psychotherapy
University Hospital Leipzig
Semmelweisstraße 10
D-04103 Leipzig
Germany
Phone: +493419725038
Fax: +493419724509
e-mail: F.Schmidt@medizin.uni-leipzig.de

For publication in: **Journal of Psychiatric Research**

Abstract

Background: The up-regulation of pro-inflammatory agents, amongst them tumor necrosis factor (TNF), may represent low-grade inflammation in major depression. To further elucidate inflammatory mechanisms related to TNF in depression, the aim of the current study was to investigate the involvement of ligands and receptors of the TNF/TNF-receptor-superfamily yet un- or little explored in major depression.

Methods: Serum levels of ligands (TNF, TNF-related weak inducer of apoptosis [TWEAK], B-cell activating factor [BAFF], tumor necrosis factor superfamily member 14 [TNFSF14; LIGHT], A proliferation-inducing ligand [APRIL]) and receptor molecules (TNF receptor superfamily member 8 [TNFRSF8; sCD30], soluble TNF receptor type 1 [sTNFR1] and type 2 [sTNFR2]) of the TNF/TNF-receptor-superfamily were measured in 50 unmedicated patients suffering from major depression and 48 healthy controls and were reassessed in 37 of the depressed patients two weeks after the initiation of antidepressive treatment.

Results: In comparison to the healthy controls, the interrelated serum levels of TWEAK, BAFF, TNFSF8, sTNFR1 and sTNFR2 were reduced both in the unmedicated and medicated depressed patients. Serum levels of BAFF and TNF significantly increased during the initiation of antidepressive treatment. In the combined sample of unmedicated depressed and healthy controls, but not the separate groups, scores of the BDI-II inversely correlated with levels of TWEAK, BAFF, sTNFR1, sTNFR2 and TNFSF8.

Conclusion: The current findings give evidence for a role of the TNF/TNF-receptor-superfamily in the pathophysiology of major depression that may involve reduced tissue regeneration and neurogenesis rather than an acceleration of pro-inflammatory pathways.

Key words: cytokines, inflammation hypothesis, major depression, TNF superfamily

1. Introduction

A low-grade inflammation with an up-regulation of inflammatory cytokines is believed to be related to the etiopathogenesis of major depression, potentially its course and treatment outcome (Leonard, 2018; Bufalino et al., 2013; Köhler et al., 2018; Schmidt et al., 2014; Schmidt et al., 2016). As a central component of the innate immune system and as one the most frequent cytokines investigated in major depression (Köhler et al., 2017), tumor necrosis factor (TNF; tumor necrosis factor ligand superfamily member 2 [TNFSF2]) may be involved in major depression not just by activating certain cascades of inflammation but by a series of mechanisms: TNF induces the indolamine-2,3-dioxygenase (IDO), which mediates the shift of metabolism of tryptophan away from 5-hydroxytryptamine (5-HT) to kynurenine, and the increased transformation of kynurenine to neurotoxic quinolinic acid (Leonard, 2018; Lichtblau et al., 2013); TNF mediates the depletion of the active fraction of 5-HT by activating serotonin transporter (Malynn et al., 2013), disrupts the negative feedback loop of the hypothalamic-pituitary-adrenal- (HPA) -axis by stimulating the excessive release of corticotrophin-releasing hormone (CRH) (Zunszain, 2011), contributes to increased redox signaling of nitric oxide (NO) (Moylan et al., 2014) but at the same time, is involved in hippocampal neurogenesis (McCoy and Tansey, 2008).

TNF is eponymous for the TNF/TNF-receptor-superfamily (TNF/TNFRSF), which consists of ligands with functional and structural similarities to TNF and receptors that contribute to inflammation, cell growth, survival or death (Croft et al., 2013). Except for few studies showing increased levels of the soluble forms of the TNF-receptors (sTNFR1 and sTNFR2) (Grassi-Oliveira et al., 2009; Papakostas et al., 2013; Diniz et al., 2010), data on components of the TNF/TNF-receptor-superfamily, such as the ligands TNFSF12 (= TNF-related weak inducer of apoptosis [TWEAK]), TNFSF13 (= A proliferation-inducing ligand [APRIL]), TNFSF13B (= B-cell activating factor [BAFF] and TNFSF14 [LIGHT], or the TNF-receptor TNFRSF8 [= sCD30], are scarce or lacking in major depression.

Given the paucity in studies reporting on parameters of the TNF/TNFRSF other than TNF and the central involvement of TNF family members in inflammatory, apoptotic as well as neuroprotective processes related to major depression, the aim of the current study was to exploratory compare serum levels of hitherto un- and little explored components of the TNF/TNFRSF between subjects with major depression and non-depressed controls and, hence, to give further evidence for a disruption of the immune system in major depression.

2. Methods

2.1. Subjects and overall design

The sample of 50 antidepressant-free depressed patients consisted of depressed in- and outpatients consecutively recruited between 02/2012 and 12/2016 from the Department of Psychiatry and Psychotherapy of the University Hospital Leipzig as part of the VIRAP ('Vigilance Regulation as Response Predictor in Antidepressant Therapy; Schmidt et al., 2017) project. Measurements were repeated in 37 patients 14 ± 1 days following the onset of antidepressant treatment (T2). At both points of measurement a German version of a structured interview (Williams et al., 1988) was performed to assess the Hamilton Depression Rating Scale (HAMD-17; Hamilton, 1960) and the Inventory of Depressive Symptomatology (IDS; Rush et al., 1996). Antidepressant therapy within two weeks of assessment consisted of a monotherapy with either escitalopram or mirtazapine. Data sets of 48 non-depressed controls were selected from a pool of participants recruited as part of the 'OBDEP' research project (Obesity and Depression: pathogenetic role of sleep and wakefulness regulation, motor activity level and neurochemical aspects; Schmidt et al., 2014). For this project, 304 participants were initially recruited from the outpatient clinic of the Integrated Research and Treatment Centre for Adiposity Diseases Leipzig (IFB), from the Department of Psychiatry and Psychotherapy of the University Hospital Leipzig and via advertisements (intranet,

internet, local newspapers). The assessment of the non-depressed controls was performed in two steps: After completing a telephone screening interview, which involved collecting socio-demographic data, assessing the presence of somatic disorders and completing a checklist of the Structured Clinical Interview for DSM-IV (SCID-I) (Wittchen et al., 1997), potentially eligible participants were invited to the study center to assess in- and exclusion criteria in more detail. In both, depressed participants and healthy controls, a Beck Depression Inventory was obtained (BDI-II; Beck et al., 1996). A Structured Clinical Interview (SCID-I) was performed to support the assessment of inclusion and exclusion criteria. The diagnosis of major depression with a current episode of depression represented an inclusion criterion for the depressed patients and an exclusion criterion for the non-depressed controls. An inclusion criterion for both groups was age ≥ 18 years. For both groups, exclusion criteria were: use of centrally active medications (including antidepressants) and non-steroidal anti-inflammatory drugs (NSAID) during the previous two weeks; serious suicide risk; organic mental disorders; use of illegal drugs and/or alcohol abuse within the past 6 months; schizophrenia, schizotypal and delusional disorders; a history of head injury with loss of consciousness exceeding 1 hour and epilepsy. For the depressed and non-depressed participants, assessments of current and past history of physical and mental health problems as well as current medication were performed using standardized forms. Patients suffering from conditions of current inflammation, inflammatory diseases and autoimmune disorders were excluded from participation. Drugs and disorders potentially affecting cytokine levels are listed in table 1. Written informed consent was obtained from all participants. The study was performed according to the Helsinki Declaration and approved by Leipzig University Ethics Committee (#278-11-22082011).

2.2. Cytokine measurements

After blood drawing, serum probes were immediately centrifuged at 3000 rpm for 10 minutes. The supernatant was aliquoted and stored in non-absorbing polypropylene tubes of 300 µl. Probes were snap-frozen in liquid nitrogen and stored at -80 °C until further measurements were conducted. TNF was measured using the Bio-Plex Pro Human Chemokine TNF- α Set, Bio Rad, Germany. All other parameters were measured with the Bio-Plex Pro Human Inflammation Assay, Bio Rad, Germany, 96-well kits that include coupled magnetic beads and detection antibodies. All analyses were performed at the Institute of Laboratory Medicine, University Hospital Ludwig-Maximilian-University Munich, Germany. The intraassay coefficient of variance (CV) of the parameters of the Inflammation Assay included into the statistical analyses was between 8.53 and 17.62 % for plate one and 1.78 and 4.66 % for plate two, the intra-assay CV for TNF was between 1.54 and 1.6. The interassay CV was between 0.15 and 6.44 %. For LIGHT, all except 10 analytes (7.4 % of the total samples) were below the lower limit of quantification (LLOQ) of 3.1 pg/ml. None of the other probes were below or above the lower (LLOQ) and upper (ULOQ) limit of quantification.

2.3. Statistics

Following the four-fold dilution of the samples volumes during the preparation process preceding the measures, all levels of all mediators were multiplied by the factor 4 before statistical analyses were conducted. Extreme outliers for each of the parameters in the total of the 135 probes were excluded for the respective analyses, identified when meeting 'value>3*interquartile range'. Following Gaussian (TWEAK, BAFF, sTNFR1, sTNFR2) and non-Gaussian distribution (APRIL, TNFRSF8, TNF) of log-transformed values, either multivariate analysis of variance (MANOVA) or generalized linear models (GLM) were performed for the analyses between groups. The following variables were included into the analyses as covariates when significantly correlating with TNF/TNF-receptor-superfamily

members and/or when they differed between the depressed and non-depressed participants: BMI, sex, smoking status, number of somatic disorders and medication. Paired t-tests or Wilcoxon signed-rank tests were performed to analyze changes in cytokine levels between the assessment points. The correlation coefficients between the parameters and severities of depression were assessed via Spearman rank correlations. The level of significance was set at $p < 0.05$ for all analyses. All analyses were performed with SPSS Version 24.

3. Results

3.1. Socio-demography

The 50 depressed patients and the 48 healthy controls did not differ in age and sex distribution, whereas more depressed patients were current smokers and the healthy controls showed a higher BMI. Both groups did not differ in the presence of somatic disorders and drugs that could potentially influence cytokine levels. Depression severities and characteristics of depression are listed in table 1.

3.2. Comparisons of levels of TNF/TNF-receptor-superfamily members between groups

Analyses between the group of unmedicated depressed subjects and the healthy subjects revealed significantly lower levels of BAFF, TWEAK, TNFRSF8, sTNFR1 and sTNFR2 in the group of depressed subjects (Table 2; Figure 1). Levels of APRIL and TNF- α did not vary significantly between the two groups. Levels of LIGHT could only be detected in 8 healthy controls ($37.28 \text{ pg/ml} \pm 26.03 \text{ pg/ml SD}$), whereas all levels within the depressed patients were below the detection range.

Comparisons between the medicated depressed patients at T2 and the healthy controls revealed significantly lower levels of BAFF, TWEAK, sTNFR1 and sTNFR2 within the depressed subjects. The two groups did not differ significantly in levels of APRIL, TNFRSF8 and TNF.

3.3. TNF/TNF-receptor-superfamily members and the clinical course, response and medication

From baseline to T2, serum levels of BAFF and TNF increased significantly within the total group of depressed patients, whereas levels of APRIL, TWEAK, TNFRSF8, sTNFR1 and sTNFR2 did not vary between the two points of time (Table 2; Figure 1).

Comparisons in cytokine levels between responders (response defined as a 40 % decrease in HAMD-17 sum score from baseline to T2; N = 14) and non-responders (N = 23) revealed significantly higher baseline levels of TWEAK in the responders ($476.52 \text{ pg/ml} \pm 119.06 \text{ pg/ml}$) versus the non-responders ($388.94 \text{ pg/ml} \pm 84.83 \text{ pg/ml}$; $p = 0.008$). Changes in levels from baseline to T2 significantly varied for TWEAK only ($F = 4.815$, $p = 0.035$), showing a slight decrease in the responders (T2: $423.10 \text{ pg/ml} \pm 202.78 \text{ pg/ml}$) and an increase in the non-responders (T2: $408.63 \text{ pg/ml} \pm 106.81 \text{ pg/ml}$).

Comparisons between patients receiving escitalopram (N = 25) and mirtazapine (N= 12) did not reveal significant differences for any of the parameters at baseline or T2. For changes from baseline to T2, no significant time*medication interaction could be observed.

3.4. Associations between levels of TNF/TNF-receptor-superfamily members and clinical variables

In the total study sample but not the separate groups, BDI-II sum scores inversely correlated with levels of BAFF ($R = -0.339$, $p < 0.001$), TWEAK ($R = -0.334$, $p = 0.001$), sTNFR1 ($R = -0.388$, $p < 0.001$) and sTNFR2 ($R = -0.467$, $p < 0.001$). Scores of HAMD-17 and IDS-C did not significantly correlate with the parameters in the unmedicated and medicated depressed patients. sTNFR1, but none of the other parameters, was higher in melancholic ($N = 28$; $2.61 \text{ ng/ml} \pm 0.66 \text{ ng/ml SD}$) versus non-melancholic depressed patients ($2.20 \text{ ng/ml} \pm 0.64 \text{ ng/ml SD}$; $p = 0.023$). Concerning the number of episodes, solely TNFRSF8 was significantly elevated in the patients with the first compared to patients with recurrent depressive episodes ($338.04 \text{ pg/ml} \pm 159.28 \text{ pg/ml SD}$; recurrent: $251.63 \text{ pg/ml} \pm 101.60 \text{ pg/ml SD}$; $p = 0.048$) and weakly correlated with the number of depressive episodes ($R = -0.285$, $p = 0.045$).

3.5. Associations between levels of TNF/TNF-receptor-superfamily members and socio-demography

In the total sample, the BMI correlated significantly with BAFF ($r = 0.310$, $p = 0.002$), sTNFR1 ($r = 0.370$, $p < 0.001$), sTNFR2 ($r = 0.545$, $p < 0.001$) and TNFRSF8 ($R = 0.219$, $p = 0.035$), but not with TWEAK, APRIL or TNF. Age correlated with APRIL ($R = 0.536$, $p < 0.001$) but none of the other mediators. APRIL, but not the other mediators, was found to be higher in the total of male participants ($30.18 \text{ ng/ml} \pm 18.92 \text{ ng/ml SD}$) compared to female participants ($19.54 \text{ ng/ml} \pm 12.79 \text{ ng/ml SD}$; $p = 0.002$) and in the healthy males ($34.59 \text{ ng/ml} \pm 21.63 \text{ ng/ml SD}$) compared to healthy females ($17.61 \text{ ng/ml} \pm 13.08 \text{ ng/ml SD}$; $p = 0.002$).

4. Discussion

In this exploratory, prospective study, differences in the levels of eight different ligands and receptors of the TNF/TNF-receptor-superfamily were investigated and compared between

depressed and non-depressed subjects. Extending findings on TNF, to the best of the authors' knowledge, this is the first investigation on the ligands BAFF, TWEAK, APRIL and LIGHT in major depression, and adds to the few studies on TNF-receptors TNFRSF8, sTNFR1 and sTNFR2 (Grassi-Oliveira et al., 2009; Papakostas et al., 2013; Diniz et al., 2010; Merendino et al., 2002). As the major results, group comparisons revealed significantly reduced means for BAFF, TWEAK, TNFRSF8, sTNFR1 and sTNFR2 in the unmedicated patients compared to non-depressed subjects, which did not normalize within the first phase of antidepressant treatment.

Seemingly, the reductions in mean levels contrast the assumption of a persistent, low grade inflammation to occur in major depression for which the TNF-system is of central importance. For instance, TNF has been shown to impair the monoamine neurotransmitter synthesis, which leads to a reduced availability for the synthesis of serotonin (Malynn et al., 2013) and an increased accumulation of neuro-toxic metabolites (Lichtblau et al., 2013). Further, TNF increase the expression and function of monoamine reuptake pumps and results in a reduced synthesis of different neurotrophic growth factors (Leonard, 2018;). Consecutively, levels of TNF and the TNF-receptors TNFRSF8, sTNFR1 and sTNFR2 were previously found increased (Köhler et al., 2017; Grassi-Oliveira et al., 2009; Papakostas et al., 2013; Diniz et al., 2010; Merendino et al., 2002). However, we could observe an overlap in levels between the depressed and healthy persons for nearly every TNF-member, especially reductions in the cytokine levels in a considerable proportion of patients. In line with this, the power of cytokines to discriminate between depression and health is yet low due to the substantial overlap in levels between acutely depressed, remitters and healthy subjects. Secondly, cytokine alterations are considered as unspecific due to the varying influence of social, psychological, biological, and medical factors (Himmerich et al., 2019). In contrast, other investigations come to the conclusion that cytokine alterations either represent a specific subtype of depression (Lotrich, 2015), that they particularly occur in sub-groups of depressed

subjects, such as atypical depression (Woelfer et al., 2019), or that they are related to some clinical features of the disorder (Schmidt et al., 2018; Euteneuer et al., 2017). Our findings that only few TNF-family members showed a relationship with the type of depression, the history of the disorder or the severity do not portend the aforementioned three assumptions. Nonetheless, given that a relevant proportion of the depressed subjects in this study exhibited reduced levels of the TNF-family members which are also involved in anti-inflammation regulation, tissue regeneration and neurogenesis, our present results challenge the assumption of a one-sided increase in cytokine levels in depression. Some features of the cytokines would indicate that also reductions in concentrations could participate in the pathogenesis of depression.

As such, BAFF is an important homeostatic signal for the B cell survival and selection, whose expression depends upon the B cell activity (Moisini and Davidson, 2009; Kreuzaler et al., 2012). One may speculate that the increase in B cells observed in depressed patients (Maes et al., 1992), accompanied with the up-regulation of BAFF receptors, leads to auto-regulatory reductions in concentrations of BAFF in major depression. On the opposite, the reduction of naïve B cells in major depression (Ahmetpahic et al., 2017) may be a consequence following the reductions in BAFF-concentrations and the missing anti-apoptotic features of BAFF.

TNFRSF8, for which the soluble sCD30 represents the extracellular fraction, is expressed by both active T and B cells and involved in proliferative and anti-apoptotic pathways as well as the control of both CD4⁺ T cell effector function and CD4⁺ T cell memory (Withers et al., 2011; van der Weyden et al., 2017). Some evidence supports our finding of a reduction of TNFRSF8 in subjects suffering from major depression: TNFRSF8 enhances resistance to the apoptosis of CD4⁺ T cells (Hirsch et al., 2010), whereas depressed patients exhibit signs of accelerated apoptosis of CD4⁺ T cells (Szuster-Ciesielska et al., 2008). In parallel to a shift in the Th2/Th1 balance towards Th1 in major depression (Komori, 2017), TNFRSF8 was found

reduced in patients with T-helper cell type 1 (Th1)-associated disorders and increased in patients with Th2-associated diseases (D'Elios et al., 1997; van der Weyden et al., 2017). Reports to compare our results with are scarce: the yet solitary investigation on TNFRSF8 in major depression reported unchanged concentrations in comparison to non-depressed subjects – however interpretations of these results are limited since only ten depressed patients had been included and the status of medication could have influenced the concentrations (Merendino et al., 2002).

For TWEAK, which modulates inflammation, cell proliferation and apoptosis (Winkles, 2008), no data on major depression yet exists. As an argument for a reduction of TWEAK to facilitate a pro-inflammatory state in major depression, TWEAK was found to attenuate the transition to adaptive Th1 immunity (Maecker et al. 2005) and to inhibit TNF-R1 signalling (Wicovsky et al., 2009). Consequently, the depletion in TWEAK may, again, lead to higher Th1 activation and reduced suppression of pro-inflammatory cytokine production (Maecker et al. 2005).

The reductions of TNF-effecting receptors sTNFR1 and sTNFR2 (McCoy and Tansey, 2008; Faustman and Davis, 2013) in the majority of the depressed patients contrast previous studies reporting on elevated levels of sTNFR2 (Papakostas et al., 2013; Diniz et al., 2010) and both sTNFR1 and sTNFR2 (Grassi-Oliveira et al., 2009; Brunoni et al., 2015). TNFR2 is critically involved in outbalancing the activity of regulatory T cells (Tregs) and T effector cells (Teffs; Ye et al., 2018; Chen and Oppenheim, 2018), and a down-regulation of TNFR2 may potentially skew T cell differentiation observed in major depression (Patas et al., 2018). Further, we may speculate that affections in concentrations of TNFR2 ~~and TNFR1~~ relates to structural and functional alterations in major depression since levels of TNFR2 were found reduced in the dorsolateral prefrontal cortex (DLPFC) in post mortem brain tissue of patients with a major depression (Dean et al., 2013). Lower TNFR2 levels were also associated with

lower activity in the ACC (Slavich et al., 2010), whose activity was found reduced in major depression (Saletu et al., 2010). Further indicative for reductions in TNFR2 at least in a parts of subjects suffering from major depression in which hippocampal function is impaired (Schmaal et al., 2016; Dietsche et al., 2014), TNF was found to have trophic effects in the hippocampus when TNFR2 was overexpressed (Yang et al., 2002), whereas TNF alone inhibits neurite outgrowth and branching (Neumann et al., 2002).

Concerning the dynamics of cytokines within the first two weeks of treatment, BAFF and TNF were found to increase. Although we could not identify a specific association with the drugs administered, there is good evidence that at least mirtazapine has pro-inflammatory properties, as it has previously been found to increase TNF-levels (Petersein et al., 2015). Following the long-term treatment, dynamics of peripheral TNF-levels were found to vary widely with a tendency towards a decrease (Köhler et al., 2017). As the changes in the majority of cytokines were not related to the clinical response here and previously (Köhler et al., 2017; Schmidt et al., 2016), the potential of cytokines as a clinically useful prediction marker seems to be small. However, as both baseline and changes in levels of TWEAK were found associated with the early response, this may be a parameter worth looking into when examining the association between members of the TNF-system and the clinical course.

Limitations of our study include that, although the BMI and the smoking habits were included as covariates into statistical analyses, an influence of the differences caused by these variables between the groups cannot entirely be ruled out. A relationship between depression severities and the majority of the parameters could be observed in the total sample but not the separate groups which could indicate a type-II-error. The results in the sub-group analyses have to be interpreted with caution, given the small sample sizes. No re-assessment of the parameters was performed in the healthy controls which could have helped interpreting the dynamics within the depressed patients. Other parameters which could underpin the arguments of reduced neuroprotective and regenerative properties of some of the investigated parameters

within major depression, such as PI3K/Akt, GSK3 or CREB, are lacking. Finally, potential mechanisms underlying the alterations in serum cytokine levels are outlined in the discussion, however, our study did not include experiments unraveling these possible mechanisms.

In conclusion, our findings of reduced serum levels of TNF/TNF-receptor-superfamily members in antidepressant-free and medicated depressed patients compared to non-depressed controls provide further insights into the role of inflammatory agents in major depression. Variation in levels may arise from changes in inflammatory cascades observed in major depression, such as a shift in the Th2/Th1 balance, an increased apoptosis of CD4+ T cells or alterations in the overall B-cells or a reduced blockade of TNF. The results may encourage further studies to provide insights into the mechanisms of the mediator actions specifically in major depression, as well as studies dealing with the interactions between antidepressants and the mediators of the TNF/TNF-receptor-superfamily.

5. Tables

Table 1: Socio-demography and clinical variables

Annotations: A = t-test, B = Chi²-test, C = Mann-Whitney-U-test

Table 2: Serum levels of TNF/TNF-receptor-superfamily members between depressed and non-depressed subjects

Annotations: A = MANOVA with 'age', 'smoking', and 'BMI' as covariates, B = generalized linear model (GLM) with 'age', 'sex', 'smoking' and 'BMI' as covariates, C = GLM with 'smoking' and 'BMI' as covariates, D = paired t-test, E = Wilcoxon test.

6. Figure

Figure 1: Serum levels of TNF/TNF-receptor-superfamily members between depressed and non-depressed subjects

Annotations: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, HC=healthy controls,

MDD_T1=unmedicated depressed subjects, MDD_T2=medicated depressed subjects

7. References

- Ahmetspahic D, Schwarte K, Ambrée O, Bürger C, Falcone V, Seiler K, Kooybaran MR, Grosse L, Roos F, Scheffer J, Jörgens S, Koelkebeck K, Dannlowski U, Arolt V, Scheu S, Alferink J. Altered B Cell Homeostasis in Patients with Major Depressive Disorder and Normalization of CD5 Surface Expression on Regulatory B Cells in Treatment Responders. *Journal of neuroimmune pharmacology the official journal of the Society on NeuroImmune Pharmacology* 2017 / 2018; 13(1):90–99.
- Beck AT, Steer RA, Brown GK. Beck Depression Inventory Second Edition Manual. San Antonio (TX): The Psychological Corporation Harcourt Brace & Company, 1996.
- Brunoni AR, Machado-Vieira R, Sampaio-Junior B, Vieira EL, Valiengo L, Benseñor IM, Lotufo PA, Carvalho AF, Ho HJ, Gattaz WF, Teixeira AL. Plasma levels of soluble TNF receptors 1 and 2 after tDCS and sertraline treatment in major depression: Results from the SELECT-TDCS trial. *J Affect Disord.* 2015(185):209–213.
- Bufalino C, Hepgul N, Aguglia E, Pariante CM. The role of immune genes in the association between depression and inflammation: a review of recent clinical studies. *Brain Behav Immun.* 2013; 31:31-47.
- Chen X, Oppenheim JJ. The phenotypic and functional consequences of tumour necrosis factor receptor type 2 expression on CD4+ FoxP3+ regulatory T cells. *Immunology* 2011; 133(4):426–433.
- Croft M, Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamilies. *Nat. Rev. Drug Discov.* 2013; 12(2):147–168.
- Dean B, Gibbons AS, Tawadros N, Brooks L, Everall IP, Scarr E. Different changes in cortical tumor necrosis factor- α -related pathways in schizophrenia and mood disorders. *Mol Psychiatry* 2013; 18(7):767–773.
- D'Elia MM, Romagnani P, Scaletti C, Annunziato F, Manghetti M, Mavilia C, Parronchi P, Pupilli C, Pizzolo G, Maggi E, Del Prete GF, Romagnani S. In vivo CD30 expression in human diseases with predominant activation of Th2-like T cells. *J Leukoc Biol.* 1997; 61(5):539–544.
- Dietsche B, Backes H, Stratmann M, Konrad C, Kircher T, Krug A. Altered neural function during episodic memory encoding and retrieval in major depression. *Hum Brain Mapp.* 2014; 35(9):4293–4302.
- Diniz BS, Teixeira AL, Talib LL, Mendonça VA, Gattaz WF, Forlenza OV. Increased soluble TNF receptor 2 in antidepressant-free patients with late-life depression. *J Psychiatr Res.* 2010; 44(14):917–920.
- Euteneuer F, Dannehl K, Del Rey A, Engler H, Schedlowski M, Rief W. Peripheral Immune Alterations in Major Depression: The Role of Subtypes and Pathogenetic Characteristics. *Front Psychiatry.* 2017; 8:250.
- Faustman DL, Davis M, Faustman DL, Davis M. TNF Receptor 2 and Disease: Autoimmunity and Regenerative Medicine. *Front Immunol.* 2013; 4(4):478.
- Grassi-Oliveira R, Brietzke E, Pezzi JC, Lopes RP, Teixeira AL, Bauer ME. Increased soluble tumor necrosis factor-alpha receptors in patients with major depressive disorder. *Psychiatry and clinical neurosciences* 2009; 63(2):202–208.
- Hamilton M. A rating scale for depression. *Journal of Neurology, Neurosurgery, and Psychiatry* 1960; 23(1):56–62.
- Himmerich H, Patsalos O, Lichtblau N, Ibrahim MAA, Dalton B. Cytokine Research in Depression: Principles, Challenges, and Open Questions. *Front Psychiatry* 2019; 10:30.
- Hirsch B, Braun FK, Al-Yacoub N, Assaf C, Kadin ME, Sterry W, Eberle J, Dürkop H. Kutanes anaplastisches großzelliges Lymphom. Apoptoseresistenz durch CD30-induzierte cFLIP-Expression. *Der Pathologe* 2010; 31(2):193–198.
- Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, Stubbs B, Solmi M, Veronese N, Herrmann N, Raison CL, Miller BJ, Lancôt KL, Carvalho AF. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr Scand.* 2017; 135:373-387.
- Köhler CA, Freitas TH, Stubbs B, Maes M, Solmi M, Veronese N, Andrade NQ de, Morris G, Fernandes BS, Brunoni AR, Herrmann N, Raison CL, Miller BJ, Lancôt KL, Carvalho AF. Peripheral Alterations in Cytokine and Chemokine Levels

After Antidepressant Drug Treatment for Major Depressive Disorder: Systematic Review and Meta-Analysis. *Molecular neurobiology* 2018; 55(5):4195–4206.

Komori T. The significance of proinflammatory cytokines and Th1/ Th2 balance in depression and action of antidepressants. *Neuropsychiatry* 2017; 7(1).

Kreuzaler M, Rauch M, Salzer U, Birmelin J, Rizzi M, Grimbacher B, Plebani A, Lougaris V, Quinti I, Thon V, Litzman J, Schlesier M, Warnatz K, Thiel J, Rolink AG, Eibel H. Soluble BAFF levels inversely correlate with peripheral B cell numbers and the expression of BAFF receptors. *Journal of immunology (Baltimore, Md. 1950)* 2012; 188(1):497–503.

Lamore R, Parmar S, Patel K, Hilas O. Belimumab (benlysta): A breakthrough therapy for systemic lupus erythematosus. *P & T a peer-reviewed journal for formulary management* 2012; 37(4):212–226.

Lichtblau N, Schmidt FM, Schumann R, Kirkby KC, Himmerich H. Cytokines as biomarkers in depressive disorder: current standing and prospects. *Int Rev Psychiatry* 2013; 25(5):592–603.

Leonard BE. Inflammation and depression: a causal or coincidental link to the pathophysiology? *Acta Neuropsychiatr.* 2018; 30(1):1-16.

Maecker H, Varfolomeev E, Kischkel F, Lawrence D, LeBlanc H, Lee W, Hurst S, Danilenko D, Li J, Filvaroff E, Yang B, Daniel D, Ashkenazi A. TWEAK attenuates the transition from innate to adaptive immunity. *Cell* 2005; 123(5):931–944.

Maes M, Stevens WJ, DeClerck LS, Bridts CH, Peeters D, Schotte C, Cosyns P. A significantly increased number and percentage of B cells in depressed subjects: Results of flow cytometric measurements. *Journal of affective disorders* 1992; 24(3):127–134.

Malynn S, Malynn S, Campos-Torres A, Moynagh P, Haase J. The pro-inflammatory cytokine TNF- α regulates the activity and expression of the serotonin transporter (SERT) in astrocytes. *Neurochem. Res.* 2013; 38(4):694–704.

McCoy MK, Tansey MG. TNF signaling inhibition in the CNS: Implications for normal brain function and neurodegenerative disease. *Journal of neuroinflammation* 2008; 2008(5):45.

Merendino RA, Di Rosa AE, Di Pasquale G, Minciullo PL, Mangraviti C, Costantino A, Ruello A, Gangemi S. Interleukin-18 and CD30 serum levels in patients with moderate-severe depression. *Mediators of inflammation* 2002; 11(4):265–267.

Merrill JT, van Vollenhoven RF, Buyon JP, Furie RA, Stohl W, Morgan-Cox M, Dickson C, Anderson PW, Lee C, Berclaz P-Y, Dörner T. Efficacy and safety of subcutaneous tabalumab, a monoclonal antibody to B-cell activating factor, in patients with systemic lupus erythematosus: Results from ILLUMINATE-2, a 52-week, phase III, multicentre, randomised, double-blind, placebo-controlled study. *Annals of the rheumatic diseases* 2016; 75(2):332–340.

Moisini I, Davidson A. BAFF: A local and systemic target in autoimmune diseases. *Clinical and experimental immunology* 2009; 158(2):155–163.

Moylan S, Berk M, Dean OM, Samuni Y, Williams LJ, O'Neil A, Hayley AC, Pasco JA, Anderson G, Jacka FN, Maes M. Oxidative & nitrosative stress in depression: Why so much stress? *Neuroscience and biobehavioral reviews* 2014; 45:46–62.

Neumann H, Schweigreiter R, Yamashita T, Rosenkranz K, Wekerle H, Barde Y-A. Tumor necrosis factor inhibits neurite outgrowth and branching of hippocampal neurons by a rho-dependent mechanism. *The Journal of neuroscience the official journal of the Society for Neuroscience* 2002; 22(3):854–862.

Papakostas GI, Shelton RC, Kinrys G, Henry ME, Bakow BR, Lipkin SH, Pi B, Thurmond L, Bilello JA. Assessment of a multi-assay, serum-based biological diagnostic test for major depressive disorder: A pilot and replication study. *Mol Psychiatry* 2013; 18(3):332–339.

Patas K, Willing A, Demiralay C, Engler JB, Lupu A, Ramien C, Schäfer T, Gach C, Stumm L, Chan K, Vignali M, Arck PC, Friese MA, Pless O, Wiedemann K, Agorastos A, Gold SM. T Cell Phenotype and T Cell Receptor Repertoire in Patients with Major Depressive Disorder. *Frontiers in immunology* 2018; 9:291.

Petersein C, Sack U, Mergl R, Schönherr J, Schmidt FM, Lichtblau N, Kirkby KC, Bauer K, Himmerich H. Impact of lithium alone and in combination with antidepressants on cytokine production in vitro. *Journal of neural transmission* 2015; 122(1):109–122.

Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): Psychometric properties. *Psychological medicine* 1996; 26(3):477–486.

Saletu B, Anderer P, Saletu-Zyhlarz GM. EEG topography and tomography (LORETA) in diagnosis and pharmacotherapy of depression. *Clinical EEG and neuroscience* 2010; 41(4):203–210.

Schmaal L, Veltman DJ, van Erp TGM, Sämann PG, Frodl T, Jahanshad N, Loehrer E, Tiemeier H, Hofman A, Niessen WJ, Vernooij MW, Ikram MA, Wittfeld K, Grabe HJ, Block A, Hegenscheid K, Völzke H, Hoehn D, Czisch M, Lagopoulos J, Hatton SN, Hickie IB, Goya-Maldonado R, Krämer B, Gruber O, Couvy-Duchesne B, Rentería ME, Strike LT, Mills NT, Zubizaray GI de, McMahon KL, Medland SE, Martin NG, Gillespie NA, Wright MJ, Hall GB, MacQueen GM, Frey EM, Carballo A, van Velzen LS, van Tol MJ, van der Wee NJ, Veer IM, Walter H, Schnell K, Schramm E, Normann C, Schoepf D, Konrad C, Zurowski B, Nickson T, McIntosh AM, Papmeyer M, Whalley HC, Sussmann JE, Godlewska BR, Cowen PJ, Fischer FH, Rose M, Penninx BWJH, Thompson PM, Hibar DP. Subcortical brain alterations in major depressive disorder: Findings from the ENIGMA Major Depressive Disorder working group. *Mol Psychiatry* 2016; 21(6):806–812.

Schmidt FM, Lichtblau N, Minkwitz J, Chittka T, Thormann J, Kirkby KC, Sander C, Mergl R, Faßhauer M, Stumvoll M, Holdt LM, Teupser D, Hegerl U, Himmerich H. Cytokine levels in depressed and non-depressed subjects, and masking effects of obesity. *Journal of psychiatric research* 2014; 55:29–34.

Schmidt FM, Sander C, Dietz M-E, Nowak C, Schröder T, Mergl R, Schönknecht P, Himmerich H, Hegerl U. Brain arousal regulation as response predictor for antidepressant therapy in major depression. *Scientific reports* 2017; 7:45187.

Schmidt FM, Schröder T, Kirkby KC, Sander C, Suslow T, Holdt LM, Teupser D, Hegerl U, Himmerich H. Pro- and anti-inflammatory cytokines, but not CRP, are inversely correlated with severity and symptoms of major depression. *Psychiatry research* 2016; 239:85–91.

Slavich GM, Way BM, Eisenberger NI, Taylor SE. Neural sensitivity to social rejection is associated with inflammatory responses to social stress. *Proceedings of the National Academy of Sciences of the United States of America* 2010; 107(33):14817–14822.

Szuster-Ciesielska A, Słotwińska M, Stachura A, Marmurowska-Michałowska H, Dubas-Slemp H, Bojarska-Junak A, Kandefer-Szerszeń M. Accelerated apoptosis of blood leukocytes and oxidative stress in blood of patients with major depression. *Progress in neuro-psychopharmacology & biological psychiatry* 2008; 32(3):686–694.

Uher R, Tansey KE, Dew T, Maier W, Mors O, Hauser J, Dernovsek MZ, Henigsberg N, Souery D, Farmer A, McGuffin P. An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. *The American journal of psychiatry* 2014; 171(12):1278–1286.

van der Weyden CA, Pileri SA, Feldman AL, Whisstock J, Prince HM. Understanding CD30 biology and therapeutic targeting: A historical perspective providing insight into future directions. *Blood cancer journal* 2017; 7(9):603.

Wicovsky A, Salzmann S, Roos C, Ehrenschröder M, Rosenthal T, Siegmund D, Henkler F, Gohlke F, Kneitz C, Wajant H. TNF-like weak inducer of apoptosis inhibits proinflammatory TNF receptor-1 signaling. *Cell death and differentiation* 2009; 16(11):1445–1459.

Williams JB. A structured interview guide for the Hamilton Depression Rating Scale. *Archives of general psychiatry* 1988; 45(8):742–747.

Withers DR, Gaspal FM, Bekiaris V, McConnell FM, Kim M, Anderson G, Lane P. OX40 and CD30 signals in CD4(+) T-cell effector and memory function: A distinct role for lymphoid tissue inducer cells in maintaining CD4(+) T-cell memory but not effector function. *Immunological reviews* 2011; 244(1):134–148.

Wittchen H-U, Zaudig M, Fydrich T, Münster RD. SKID Strukturiertes Klinisches Interview für DSM-IV. Achse I und II. *Zeitschrift für Klinische Psychologie und Psychotherapie* 1997 / 1999; 28(1):68–70.

Woelfer M, Kasties V, Kahlfuss S, Walter M. The Role of Depressive Subtypes within the Neuroinflammation Hypothesis of Major Depressive Disorder. *Neuroscience*. 2019; 403:93-110.

Yang L, Lindholm K, Konishi Y, Li R, Shen Y. Target depletion of distinct tumor necrosis factor receptor subtypes reveals hippocampal neuron death and survival through different signal transduction pathways. *The Journal of neuroscience the official journal of the Society for Neuroscience* 2002; 22(8):3025–3032.

Ye L-L, Wei X-S, Zhang M, Niu Y-R, Zhou Q. The Significance of Tumor Necrosis Factor Receptor Type II in CD8+ Regulatory T Cells and CD8+ Effector T Cells. *Frontiers in immunology* 2018; 9:583.

Zunszain PA, Anacker C, Cattaneo A, Carvalho LA, Pariante CM. Glucocorticoids, cytokines and brain abnormalities in depression. *Progress in neuro-psychopharmacology & biological psychiatry* 2011; 35(3):722–729.